PAVE 100 Viral and Host Genetics Workshop
Vaccine Research Center, NIH
July 31, 2007

Executive Summary

The PAVE 100 trial is designed to be a test-of-concept phase IIb efficacy study of the NIAID/VRC multiclade DNA/rAd vaccine product. The trial will commence, pending the appropriate approvals, in the Fall of 2007 under the auspices of the Partnership for AIDS Vaccine Evaluation (PAVE). PAVE 100 will test whether the vaccine candidate can prevent acquisition of infection or decrease plasma viremia in subjects who become infected despite vaccination. If either effect is observed, the study is designed to evaluate host immune responses and viral and host genetic parameters associated with protection.

On behalf of the PAVE partners, the U.S. Military HIV Research Program (USMHRP) and the NIAID Vaccine Research Center (VRC) sponsored a workshop to discuss approaches to viral and host genetic analyses that would be performed to support the PAVE 100 study. Approximately 30 scientists met July 31, 2007 at the Vaccine Research Center, in a day-long workshop. The overall goals of the workshop were: 1) to discuss and recommend optimal strategies to determine a genetic sieve effect, or a phylogenetic signal, in viral strains isolated from subjects with breakthrough infections, and 2) to discuss and recommend strategies to identify host genetic factors that might impact immune responses to vaccination, adverse events to vaccination, or impact the major endpoints of the trial. Key scientific issues and summary recommendations are reported here.

The workshop included three sessions. Session I was an overview of the design of the PAVE 100 study, its primary and secondary endpoints and the molecular epidemiology in the regions where the study would be conducted. Session II focused on genetic analysis of viral breakthrough infections and Session III focused on host genetic analyses. The latter two sessions included discussion of the major scientific questions to be addressed and the optimal application of technologies to generate the necessary data.

Workshop Recommendations

PAVE 100 provides a unique opportunity to evaluate the effectiveness of a candidate vaccine and to understand the virologic, immunologic and genetic influences on potential vaccine effects. Secondary and exploratory study objectives include immune correlates of protection and viral sieve analysis to determine the relationship of sequences in HIV-1 strains in breakthrough infections to the HIV-1 sequences in vaccine antigens, and in infected vaccinees vs. placebo recipients. Participants agreed that a comprehensive set of systematically collected viral and host genetic data will serve PAVE 100 and vaccine science for many years to come.

1) *Analysis of viral genetic sequences from PAVE 100 associated infections*

a) Viral genetic analysis should proceed by full genome sequencing of viral RNA derived from the first seropositive plasma of all infected individuals in the PAVE 100 study. Viral sequences from subsequent time points would add additional value, particularly if the vaccine demonstrates an effect on a specified endpoint such as viral load. Multiple sequences from selected viral genes, probably in the range of 10 – 20 per sample, will need to be generated from a given time point to appreciate the complexity of the early viral quasispecies, and its evolution over time. Including sequence analysis from known transmitters, as in the case of discordant couples, would be particularly valuable. All data should be stored in an accessible central repository. Both genetic distance and phylogenetic methods can be applied to the data analysis, as well amino acid signature analysis of sets of specified epitopes or sites Single genome analysis, performed by limiting dilution PCR amplification may be the preferred technology for sequence analysis if full length genome sequences can be achieved on a large scale. The working group recommended below will advise on optimal methodologies for sequence and data analysis.

b) There was discussion about the potential to study HIV transmission among study participants to assess if vaccination decreases subsequent transmission. This could be considered in some settings with molecular transmission studies, where the frequency of virus transmission from vaccinees vs. placebos could be compared by sequence analysis of viruses detected within relatively closed populations. This would become especially important if the main effect of vaccination is a reduction in viral load set-point; the question will then arise whether HIV transmission will be meaningfully impacted, particularly during the period of heightened viremia associated with acute infection. There was agreement that such a demonstration would be important and that a separate protocol would be needed in specific study regions to address this issue.

2) *Definition of host genetic determinants that affect vaccination.*

HLA typing and genetic analysis targeted to other known genes or gene families associated with HIV infection or disease progression can provide useful information for interpreting vaccine immunogenicity and endpoints in the PAVE 100 study, and should be performed. Currently unknown genetic associations may provide additional information and genome-wide analysis using high density SNP arrays will be needed to identify them. Thus, a coordinated assessment of HLA types, specific host genes and whole genome SNP analysis was recommended. In particular, it was recommended that high density SNP analysis be performed on all study participants and the data maintained in a database to allow analysis of associations with PAVE 100 study outcomes. Since methodologies for SNP analysis and HLA typing are evolving, the PAVE 100 protocol team should be advised by an expert working group that will recommend optimal methodologies and data analysis plans. Supplemental funding is needed for this effort, which is a high priority.

3) *Formation of a PAVE 100 working group on viral and host genetic studies.*

a) The PAVE 100 study and its associated analyses are complex. The clinical study has primary, secondary and exploratory objectives, there are numerous vaccine antigens and there is substantial viral and human genetic diversity included in the study. The PAVE 100 protocol team would benefit from the recommendations of an expert working group to provide advice on the key scientific questions and optimal technical and analytical approaches to viral and host genetic studies in the context of the PAVE 100 trial.

b) Due to the varied expertise needed to integrate viral and host genetic analysis with the virologic and immunological endpoints of PAVE 100, and the complex clinical aspects of the study, it is recommended that a viral and host genetics working group be comprised of scientists with specific expertise in viral and host genetics, and should include immunologists, clinicians and bioinformaticists. The working group would also advise on the specific PAVE 100 supplementary protocols needed to support the recommended genetic studies discussed here.

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Summary of Workshop Sessions

Overview of PAVE 100

The Partnership for AIDS Vaccine Evaluation (PAVE), a consortium of U.S. government and U.S. government-funded organizations stands poised to launch its first Phase IIB, test-of concept efficacy trial, PAVE 100. The candidate vaccine delivers multiple HIV-1 genes including env genes from three genetic subtypes and gag, pol, and nef from subtype B, in a DNA-prime, recombinant adenovirus (rAd)-boost regimen. PAVE 100 will be conducted by four partner organizations: The HIV Vaccine Trials Network (HVTN), The International AIDS Vaccine Initiative (IAVI), USMHRP, and the Centers for Disease Control and Prevention (CDC). The study will enroll approximately 8500 volunteers partitioned as follows: ~ 3000 Americas, ~ 2500 southern Africa, ~ 3000 East Africa. The primary trial endpoints are acquisition of HIV-1 infection and plasma viral load measured within 6 months after infection. Based on an endpoint driven design, 180 HIV-1 infections at week 26 or later will provide a 90% power to determine a vaccine efficacy of 0.42 for acquisition and a 0.4 Log_{10} reduction in viral load. During the follow up of study participants, over 300 acquisition events are expected to occur by the end of the third or fourth year.

Secondary objectives of the trial include an evaluation of vaccine immunogenicity in a subset of vaccinees, an evaluation of immune correlates of protection, and evaluations of vaccine immunogenicity and efficacy in vaccinees based on gender, geographic region and on pre-existing neutralizing titers to Adenovirus serotype-5. Exploratory objectives include a viral sieve analysis to determine the relationship of sequences in HIV-1 strains in breakthrough infections to the HIV-1 sequences in vaccine antigens, and in infected vaccinees vs. placebo recipients, as well as evaluation of potential vaccine effects on CD4 count trajectories, CD4 memory cell preservation and time to disease progression. A number of other ancillary studies are also under discussion.

Viral Genetic Analysis

Francine McCutchan and Peter Gilbert noted that, compared to viral sieve analysis for the VaxGen trial which included only Env gp120 from clade B, the PAVE 100 vaccine includes antigens from more viral genes: gag, pol, nef and from three clades of env. The number of genes and clade diversity by region of the study add complexity to the sieve analysis and can affect the power to detect a sieve affect associated with vaccine efficacy (VE). Viral sieve analysis can use a genetic distance approach or phylogenetic methods and both were discussed. Peter Gilbert presented power calculations based on genetic distances using full genome sequence data and based on specified known viral regions – such as known CTL epitopes. The latter approach, based on amino acid signature analysis, may have greater power to detect sieve effects. High-

dimensional data hypothesis testing and prediction methods can be used to identify amino acid patterns that discriminate vaccine from placebo infecting sequences. The potential limitations in power will require further discussion and analysis. Bette Korber presented phylogenetic approaches to viral genetic analysis that were based on experience from recent studies of acute vs chronic infection evaluating Env sequences. The use of phylogenetic methods permits correction for signatures pertaining to specific lineages, which could otherwise be interpreted as signatures of a vaccine effect.

There was agreement that full genome viral sequencing should be obtained from the first seropositive plasma samples from each breakthrough infection. Full-length viral sequences from subsequent time points would add additional value, particularly if the vaccine demonstrates an effect on a specified endpoint such as viral load. Multiple sequences from selected viral genes, probably in the range of 10 – 20 per sample, will need to be generated and analyzed from a given time point to appreciate the complexity of the early viral quasispecies, and its evolution over time. Sequence data need not be generated by one single laboratory, but all data should be stored in an accessible central repository. There was additional discussion about the potential importance of limiting dilution techniques for viral genome PCR amplification. Single genome amplification (SGA) methods are now commonly used for env and gag gene PCR, and their application to full genome PCR amplification is under development. SGA will be more costly than non-SGA methods, but its application, wherever feasible, would build upon a growing body of data developed through this approach. Further discussion focused on related immunogenicity and host genetics data that would be needed to develop a full viral sieve analysis plan. Such data include vaccine induced T-cell and humoral immune responses and host HLA typing. The latter would be included in the characterization of CTL epitopes that will be mapped and analyzed as part of the viral sieve analysis.

Jim Mullins and Cynthia Derdeyn presented data on the evolution of viral quasispecies during acute infection. Both T-cell and antibody responses could affect viral evolution, and the kinetics of the sequence evolution could affect the data derived from the first sampled time point in PAVE 100 – which will be within a 6 month window. There was agreement that additional data on the kinetics and extent of viral sequence evolution during early infection are needed and should be considered as part of the PAVE 100 analysis plan.

Specific decisions about the specific viral sieve and phylogenetic analysis plans will require additional discussion with the PAVE 100 protocol team. A PAVE 100 working group made up of experts in this area is recommended. This working group should advise on optimal methodologies and on approaches for data analysis. This working group should consider methods to address the potential impact of viral genetic diversity and multiple subtypes on these analyses, and methods to improve the power to detect genetic effects associated with VE.

There was also discussion about the potential to study HIV transmission among study participants to assess if vaccination decreases subsequent transmission. This would become especially important if the main effect of vaccination is a reduction in viral load set-point; the question will then arise whether HIV transmission will be meaningfully impacted, particularly during the period of heightened viremia associated with acute infection. There was agreement that such a demonstration would be important and that expanded study designs would have to be considered to address this issue. Community studies, with a detailed analysis of social interactions, could be considered as an approach to answer this question. Further discussion will be needed to determine whether PAVE 100 can be leveraged in the context of certain cohorts (discordant couples, high risk CSW or MSM cohorts, newly?-infected women who become pregnant) to approach this question.

Host Genetic Analysis

A range of approaches are available to investigate host genetic effects on various aspects of PAVE 100: the infection endpoint, set-point viral load, the immune response to vaccination, vaccine-associated adverse reactions, and the intersection of these outcomes. These were discussed during presentations by David Goldstein, Mary Carrington, Daniel Geraghty and Francine McCutchan. The most comprehensive approach is whole genome SNP mapping, where SNP "tags" throughout the human genome are used to detect associations with a given variable, such as HIV infection or stratified viral load. Recent experience with a large European cohort, investigated with an Illumina chip that can distinguish >550,000 polymorphisms, found significant associations with set-point viral load in HIV infection near HLA B and C loci, including the HLA B57 allele. This study illustrates the power of genome-wide SNP mapping to fortify known associations and to discover previously unknown genetic factors that can impact vaccine trial endpoints, and potentially explain their plausible mode of action. One aspect of the Illumina chip important for PAVE 100 is the possibility of lower coverage for populations other than those of European ancestry. The expanded 1 million SNP chip expands coverage for African populations, though some limitations remain. There was general agreement regarding the use of newer high density chip arrays to explore host genetic associations in PAVE 100.

Additional discussion was devoted to targeted genetic approaches, including direct sequence analysis of HLA and KIR, and their impact on HIV disease. Several HLA associations with disease progression are well known and various alleles can have different temporal effects, some impacting early infection and others having a later effect. There may also be interactions between KIR and specific HLA types. Therefore, coordinated assessment of specific host genes can address and expand on the known genetic associations and provide complementary information to that gained by whole-genome SNP analysis. Several approaches to HLA typing were discussed including the use of SNP arrays, real-time PCR amplification of HLA exons and sequence-based approaches that incorporate more complete evaluation of the target genes. The potential advantages and feasibility of each approach will have to be considered by the PAVE 100 team. Since methodologies for SNP analysis and HLA typing are evolving rapidly, there was agreement that the PAVE 100 protocol team should be advised by an expert working group that would recommend optimal methodologies and data analysis plans. A combined working group for viral host genetics was suggested, as there are overlapping areas of discussion and expertise.

Discussion ensued as to the optimal range and scope of human genetic studies in PAVE 100. Genome wide SNP analysis and targeted genetic typing could be applied to the entire PAVE 100 study population to assess genetic markers associated with vaccine side effects, and more focused analysis could be done for associations with immunogenicity and protective effects of the vaccine. Analysis of breakthrough infections in a case-control format, and participants in the immune sub-study were mentioned as highly valuable sub-components, but evaluation of all trial participants may be feasible, valuable, and limited only by cost considerations, and would significantly increase the value of archived PBMCs from subjects not involved in the immunogenicity assessment or case-matched control of breakthrough infection analysis. The challenge remains to find the best combination of host genetic evaluations, and their target populations, but it is clear that the tools are in place, and enthusiasm is high, to incorporate human genetic analyses into PAVE 100.

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